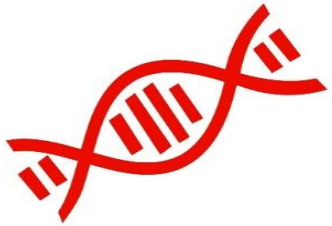


Sol Ruiz, PhD, Spain

- Head of Biologics, Biotechnology and Advanced Therapies, Spanish Medicines Agency, Spain

GaBI
Educational
Workshops

24 September 2019, Ankara HiltonSA, Turkey

2nd Turkish Interactive Workshop on
REGULATORY ASSESSMENT OF BIOSIMILARS

Regulatory aspects of biosimilars in the EU

Sol Ruiz, PhD
24 September 2019

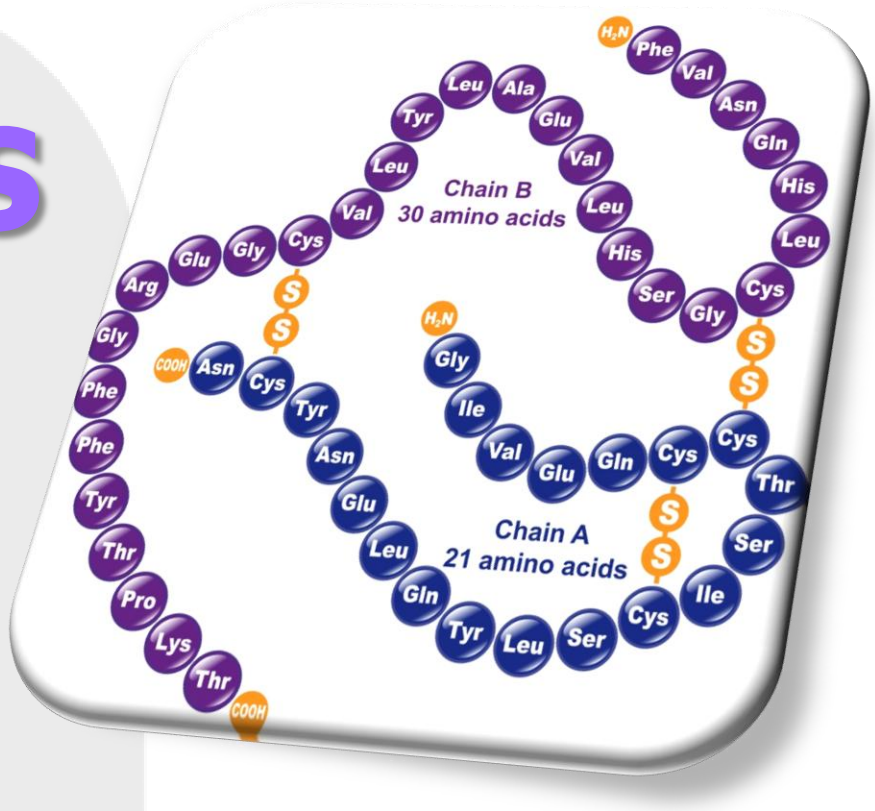
GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

PPCI
Pro Pharma
Communications
International

MINISTERIO
DE SANIDAD, CONSUMO
Y BIENESTAR SOCIAL

agencia española de
medicamentos y
productos sanitarios

Biosimilars in the EU



Sol Ruiz

2nd Turkish Interactive Workshop on
REGULATORY ASSESSMENT OF BIOSIMILARS
24 September 2019

How to establish biosimilarity?

1. extensive characterization
(physico-chemical-biological)

***2. in vitro* functional activity**
(mechanism of action of the molecule)

***3.* efficacy and safety studies**
('best' population)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

2015

Quality

- **Same aa sequence**, posology and route of administration
- Strength, pharmaceutical form, and formulation may differ
- Molecular differences to enhance efficacy not allowed

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 May 2014
EMA/CHMP/BWP/247713/2012
Committee for Medicinal Products for Human Use (CHMP)

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)

Date for coming into effect	1 December 2014

physicochemical properties

Evaluation of physicochemical parameters;

- composition
- physical properties
- **primary and higher order structures**
- structural identification of **product-related substances** and **impurities** (stress and accelerated stability studies)

Biologicals

due to its complexity it cannot be fully characterized by analytical testing alone

quality determined by a combination of *physico-chemical and biological testing*, together with the *production process and its control*

biological activity and immunogenicity are dependent upon all its *structural features*

COMMISSION DIRECTIVE 2003/63/EC of 25 June **2003**

"The process is the product"

HOWEVER...

Is it possible to establish comparable Efficacy & Safety between products derived from two different manufacturing processes?

- **Changes in the manufacturing process**
- **Biosimilarity**

Is it possible to establish comparable Efficacy & Safety between products derived from two different manufacturing processes?

- **Changes in the manufacturing process**
- **Biosimilarity**

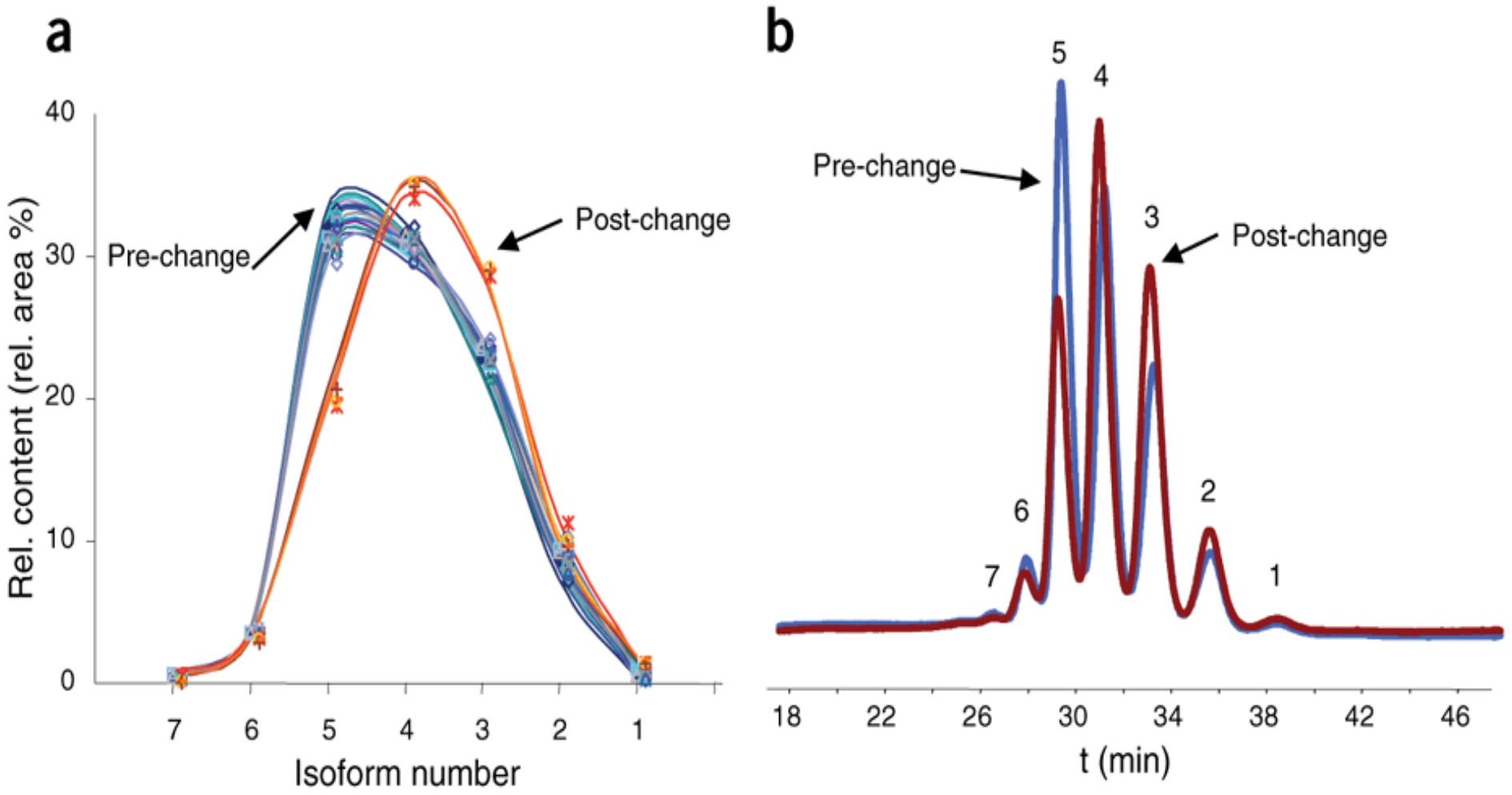


Figure 1 Comparison of the pre- and post-change Aranesp batches measured by capillary zone electrophoresis. (**a**) Relative content of the individual isoforms of the pre-change ($n = 18$) and the post-change ($n = 4$) batches. (**b**) Representative electropherograms; peaks are labeled with the isoform number.

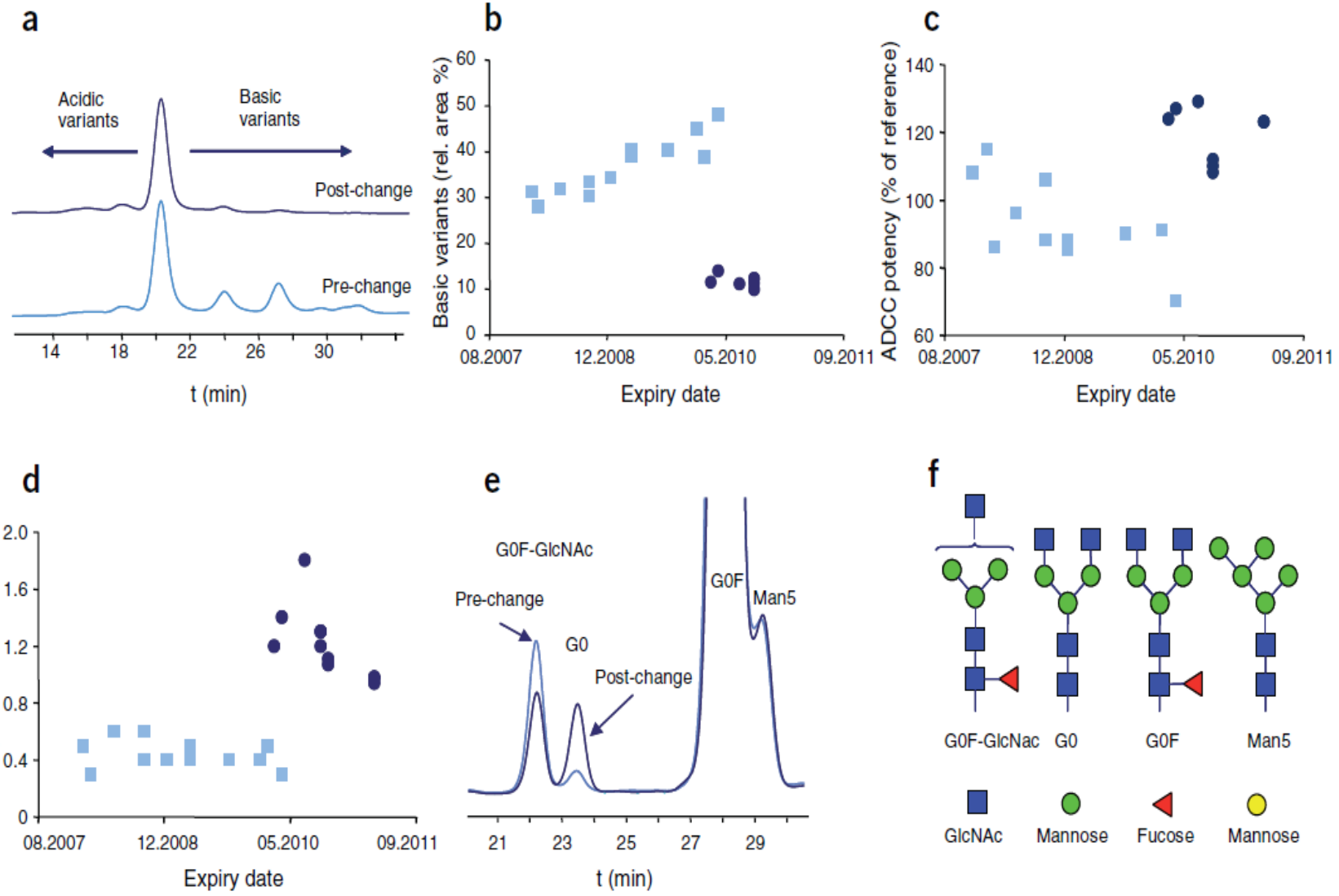


Figure 2 Comparison of the different pre- and post-change batches of Rituxan/Mabthera. (**a**) Exemplary CEX chromatograms. (**b**) Amount of basic variants of the pre-change ($n = 12$) and post-change ($n = 6$) batches as measured by CEX. (**c**) ADCC potency of the pre-change ($n = 11$) and post-change ($n = 8$) batches. (**d**) Relative amount of the G0 glycan of the pre-change ($n = 13$) and post-change ($n = 11$) batches. (**e**) Exemplary glycan mapping chromatogram (**f**) Glycan legend.

Subsequent post Marketing Authorisation applications agreed upon are summarised in the table below:

Scope	Application number	Type of modification[1]	Notification/ Opinion issued on[2]	Commission Decision Issued/amended on
Update of or change(s) to the pharmaceutical documentation	II/011	II	10.08.01	N/A
Update of Summary of Product Characteristics and Package Leaflet	II/12	II	01.03.01	27.06.01
Minor change in labelling or package leaflet not connected with the SPC (Art. 61.3 Notification)	N/13	N	10.08.01	03.10.01
Extension of Indication	II/14	II	18.10.01	21.03.02
Update of the SPC based on efficacy and safety data	II/16	II	24.04.02	15.07.02
Renewal	R/17	R	25.04.03	28.07.03
Change in the batch size of finished product	I/18	I	25.04.03	N/A
Minor changes in manufacture of the medicinal product	I/19	I	25.04.03	N/A
Change in test procedure of active substance	I/20	I	25.04.03	N/A
Change in test procedure of active substance	I/21	I	25.04.03	N/A
Withdrawal of the manufacturing authorisation for a site of manufacture	I/22	I	20.06.03	N/A
Change in or addition of manufacturing site(s) for part or all of the manufacturing process	I/23	I	25.06.03	N/A
Quality changes	II/25	II	25.09.03	14.01.04
Replacement of an exipient with a comparable excipient	I/026	I	21.08.03	N/A
Change(s) to the manufacturing process for the active substance	II/27	II	26.02.04	05.03.04
Update of Summary of Product Characteristics	II/28	II	21.01.04	19.03.04
Update of Summary of Product Characteristics	II/29	II	21.01.04	19.03.04
Change(s) to the manufacturing process for the active substance	II/30	II	03.06.04	11.06.04
Extension of Indication	II/31	II	23.06.04	02.08.04
Minor change in labelling or package leaflet not connected with the SPC	N/32	II	08.06.04	-

Is it possible to establish comparable Efficacy & Safety between products derived from two different manufacturing processes?

YES

- Changes in the manufacturing process

- **Biosimilarity**

IMPROVED PRODUCTION PROCESS

- Use of well known and extensively characterized cell lines
- Wide experience with purification processes
- Increased process knowledge ("established conditions") ➔ robustness, reproducibility

IMPROVED ANALYTICAL METHODS

high sensitivity and reproducibility:

- better characterization tools
- tighter release specifications

Table 1. Summarized attributes and key findings.

Category	Product Quality Attributes	Analytical Methods	Assessment
		Physicochemical characterization	
Primary Structure	Molecular weight	Intact mass under reducing/non-reducing conditions	Similar to RP
	Amino acid sequence Terminal sequence Methionine oxidation Deamidation C-terminal and N-terminal variants	Peptide mapping by LC-ESI-MS/MS using a combination of digestion enzymes	Similar to RP
	Disulfide linkage mapping	peptide mapping under non-reducing condition	Similar to RP
Higher-order Structure	Protein secondary and tertiary structure	Far- and near-UV CD spectroscopy, ITF, HDX-MS, Antibody conformational array, DSC	Similar to RP Similar to RP Similar to RP
Glycosylation	N-linked glycosylation site determination	LC-ESI-MS/MS	Similar to RP
	N-glycan identification	Procainamide labeling and LC-ESI-MS/MS	Minor differences were observed, but not clinically meaningful
	N-glycan profile analysis	2-AB labeling and HILIC-UPLC	Similar in terms of %Afucose+%HM and %Gal, %Charged glycans of SB2 is lower, but not clinically meaningful
Aggregation	Soluble aggregates	SEC-UV, SEC-MALLS/RI SV-AUC	Slightly higher compared with RP in HMW analyzed by SEC/UV, but SV-AUC and SEC-MALLS profiles of SB2 was similar to that of RP
Fragmentation	Low molecular weight	Non-reduced CE-SDS Reduced CE-SDS	Similar to RP
Charge heterogeneity	Acidic variants Basic variants	CEX-HPLC and icIEF	Similar to RP Lower compared with RP, but not clinically meaningful
		Biological Characterization	
Fab-related Biological Activity	TNF-α neutralization activity	TNF-α neutralization assay by NF-κB reporter gene assay	Similar to RP
	TNF-α binding activity	FRET	Similar to RP
	Apoptosis activity	Cell-based assay	Similar to RP
	Transmembrane TNF-α binding assay	FACS	Similar to RP
Fc-related Biological Activity	FcRn binding	AlphaScreen®	Similar to RP
	FcγRIIIa (V/V type) binding	SPR	Similar to RP
	ADCC using healthy donor PBMC	Cell-based assay	Similar to RP
	CDC	Cell-based assay	Similar to RP
	C1q binding	ELISA	Similar to RP
	FcγRIa binding	FRET	Similar to RP
	FcγRIIa binding	SPR	Similar to RP
	FcγRIIb binding	SPR	Similar to RP
	FcγRIIIb binding	SPR	Similar to RP

analysis of the primary structure (aa sequence)

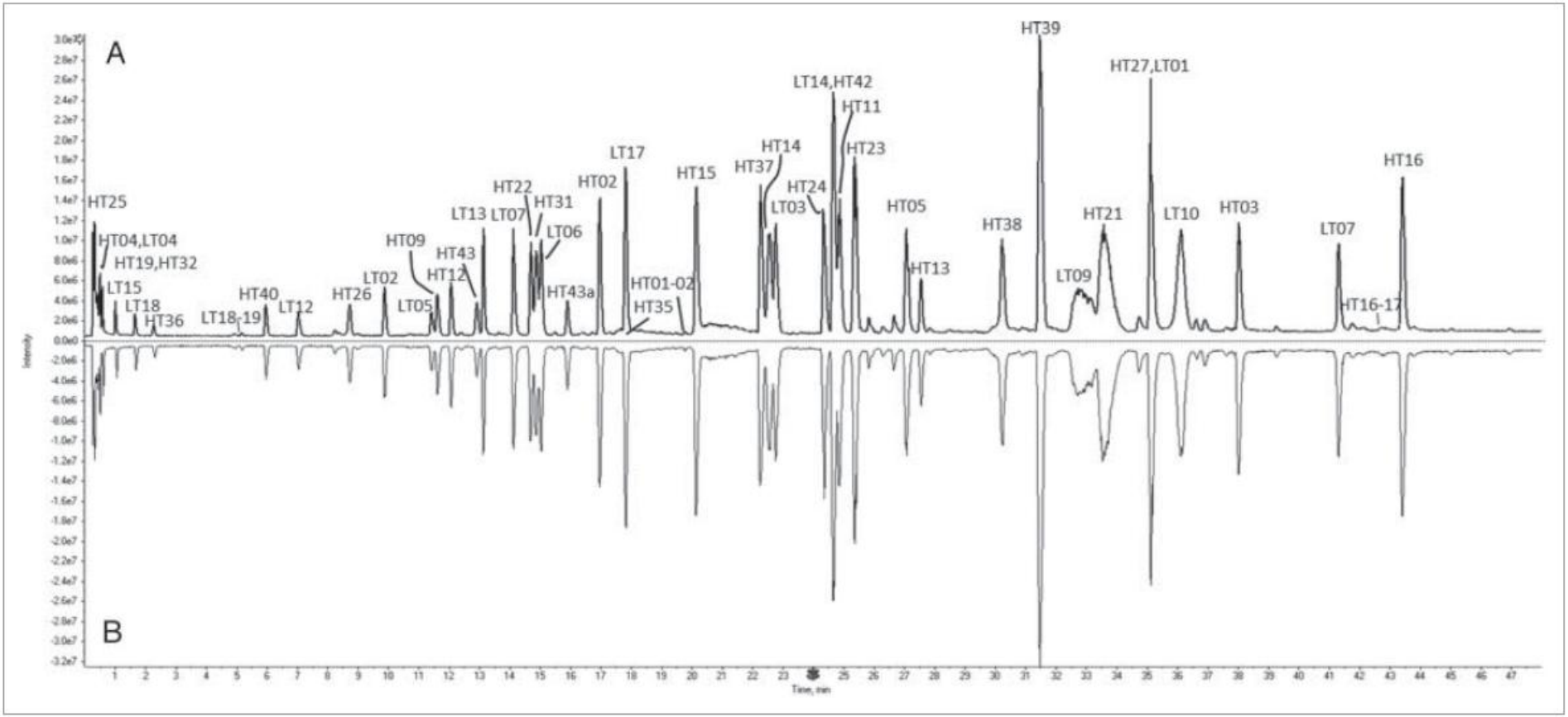


Figure 2. Comparison of Total Ion Chromatogram of LC-ESI-MS peptide mapping between (**A**) CT-P13 and (**B**) RMP, and peptide peak assignment.

analysis of the secondary structure (folding)

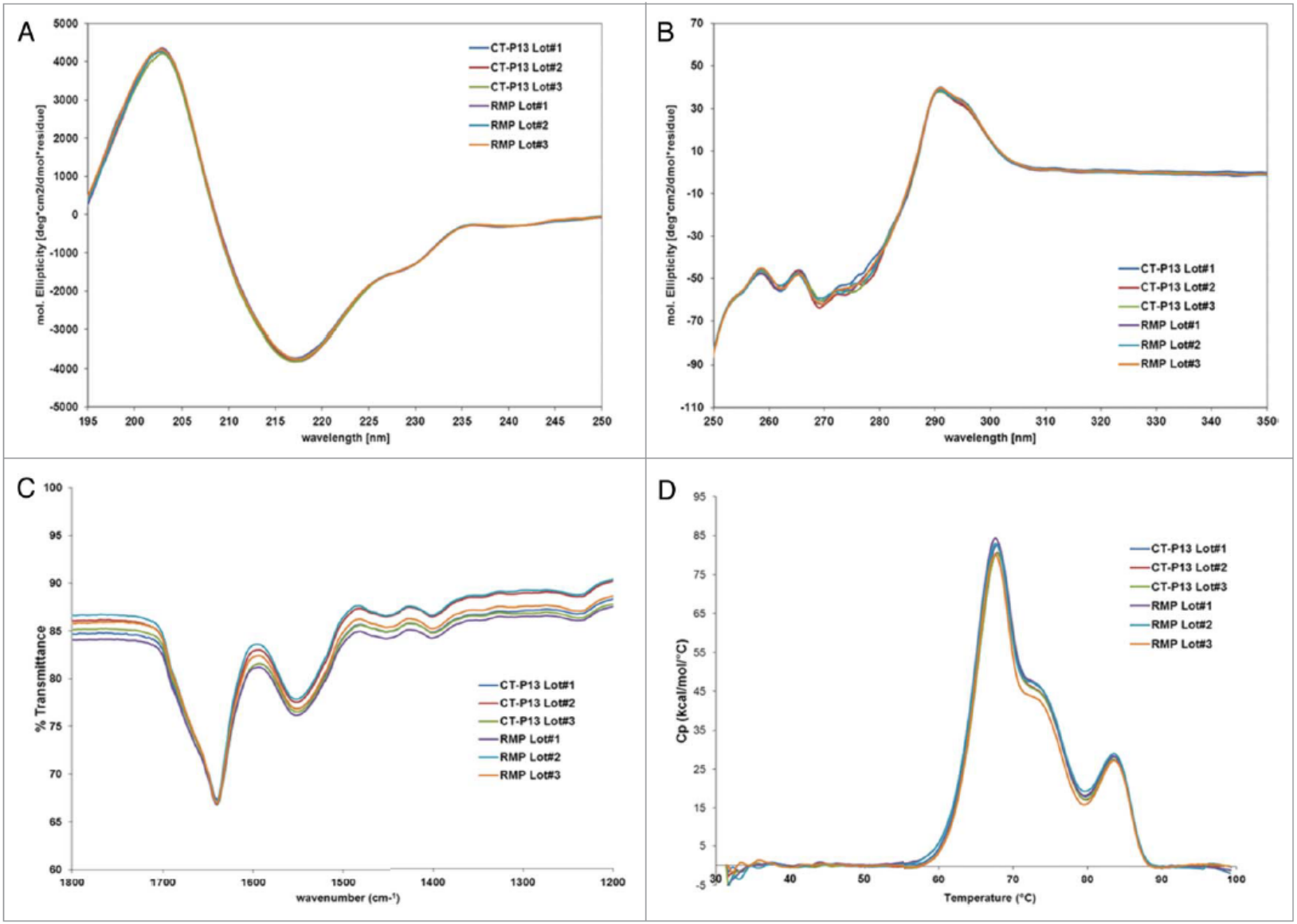


Figure 3. Higher order structure analysis: (**A**) Far-UV CD; (**B**) Near-UV CD; (**C**) FT-IR; (**D**) DSC.

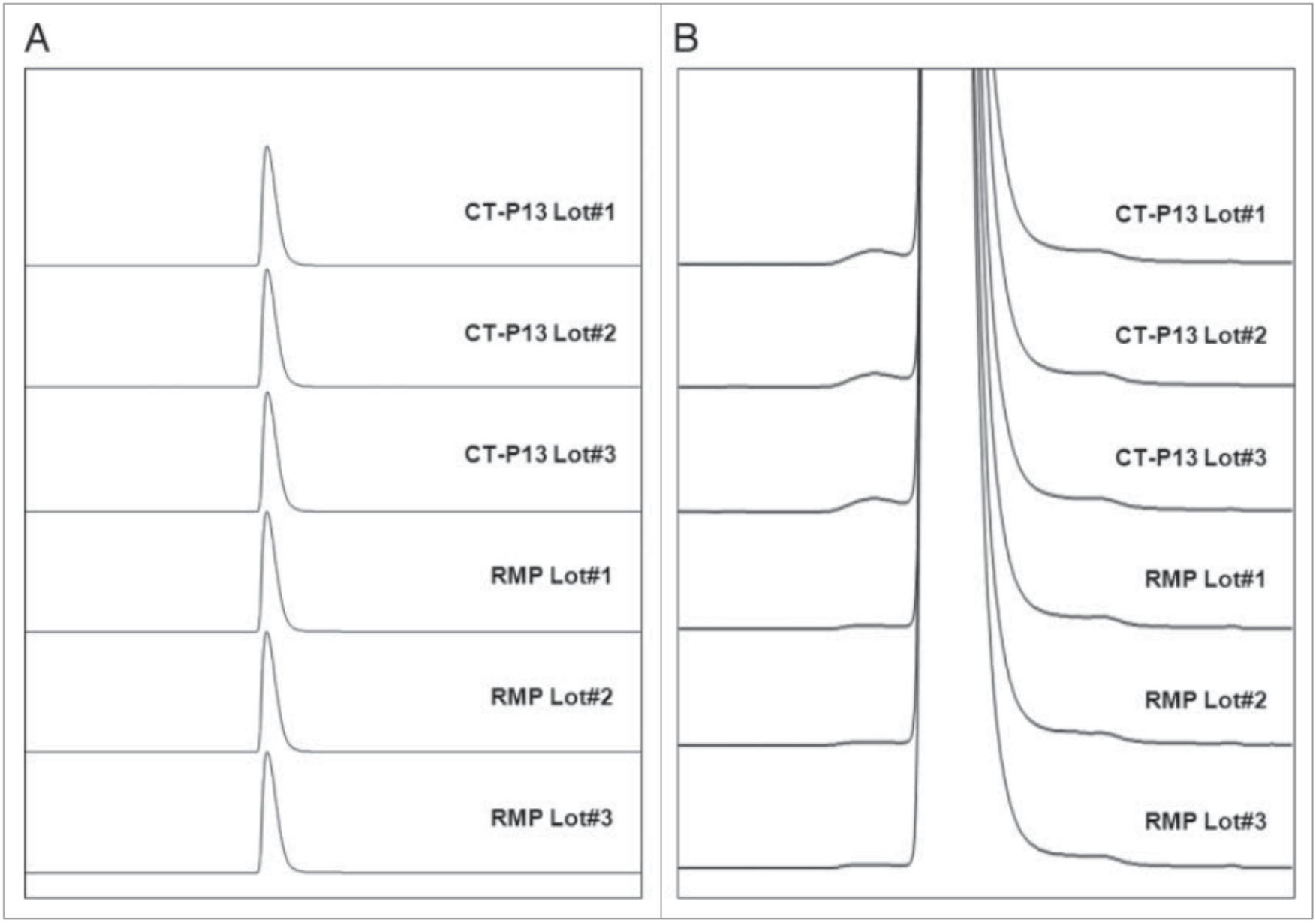


Figure 6. Comparison of Size Exclusion Chromatographies between CT-P13 and RMP: (**A**) Full scale view of chromatograms; (**B**) Expanded view of chromatograms.

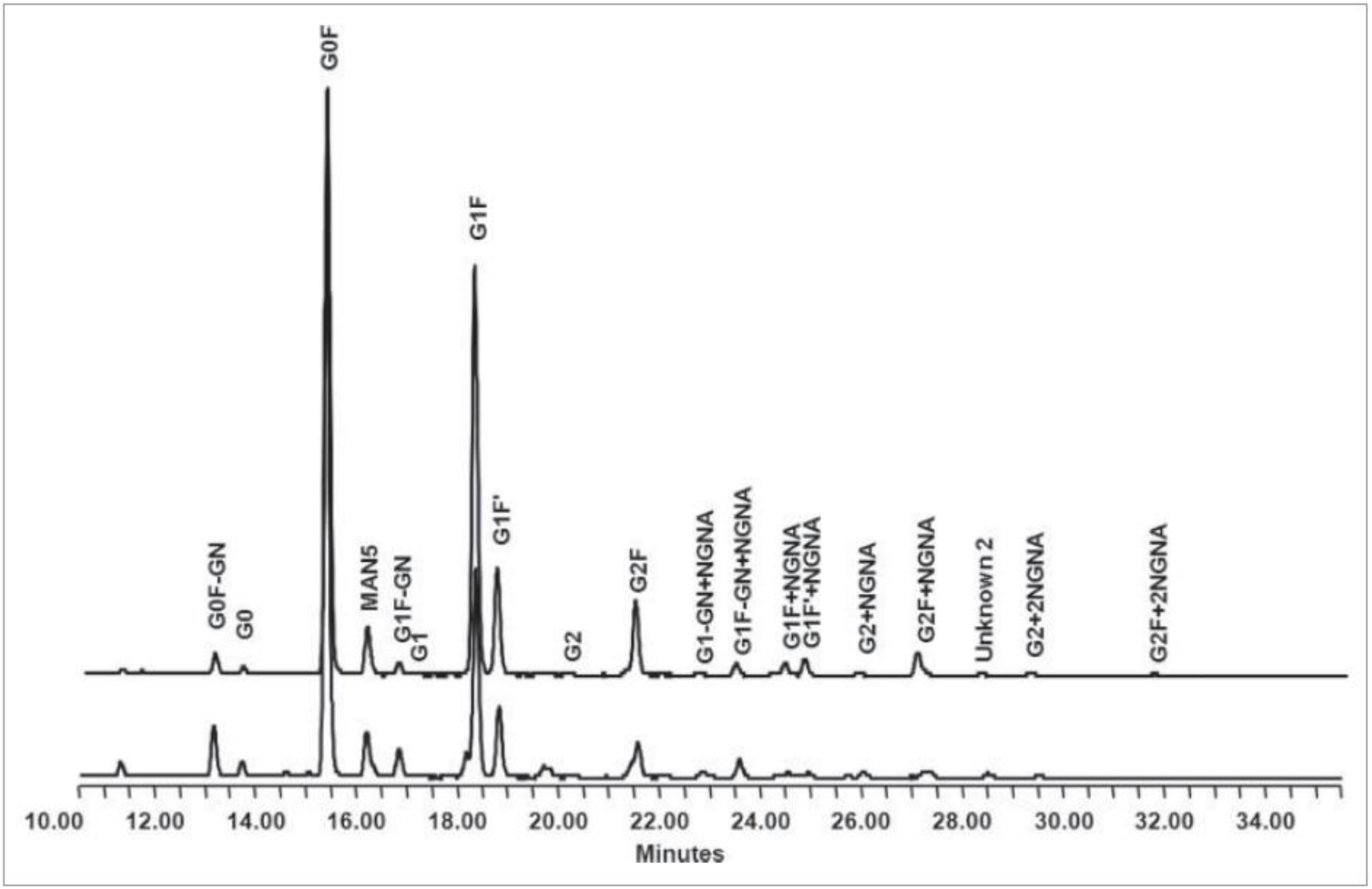
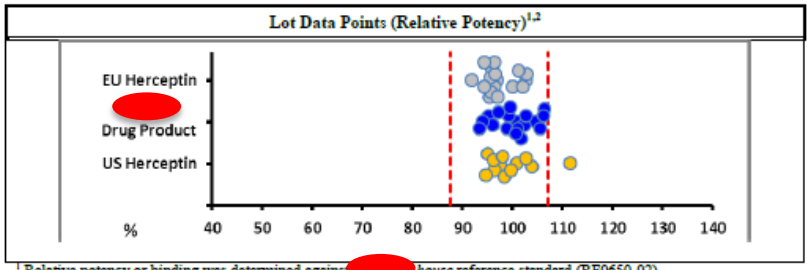


Figure 11. Comparison of oligosaccharide profiles between RMP (up) and CT-P13 (down) analyzed by normal phase chromatography.

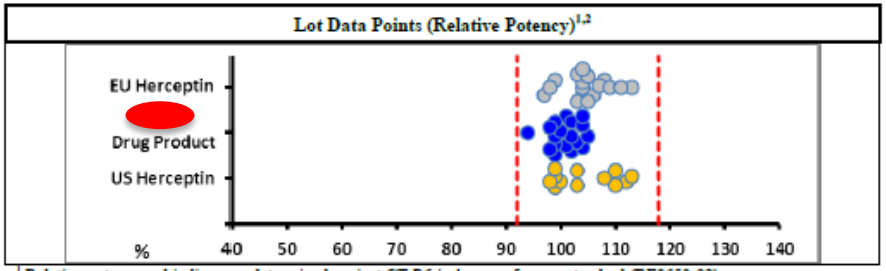
Table 3. Summary of binding affinity and in vitro potency results

Test items	CT-P13 Average (%) (Range)	SD	RMP Average (%) (Range)	SD	TOST	Method
TNF binding	99 (97–105)	2.5	100 (94–104)	2.8	<0.0001	ELISA
FcRn	101 (95–109)	4.2	97 (93–103)	3.3	<0.0001	SPR
C1q	100 (91–116)	6.6	98 (87–109)	8.2	<0.0001	ELISA
TNF binding	101 (92–110)	6.0	100 (90–112)	7.1	<0.0001	Cell-based
TNF Neutralization	102 (95–107)	4.7	104 (98–110)	2.9	<0.0001	Cell-based
Apoptosis	101 (91–105)	5.0	101 (92–110)	2.5	<0.0001	Cell-based
CDC	102 (91–116)	8.0	93 (84–115)	8.0	0.0011	Cell-based



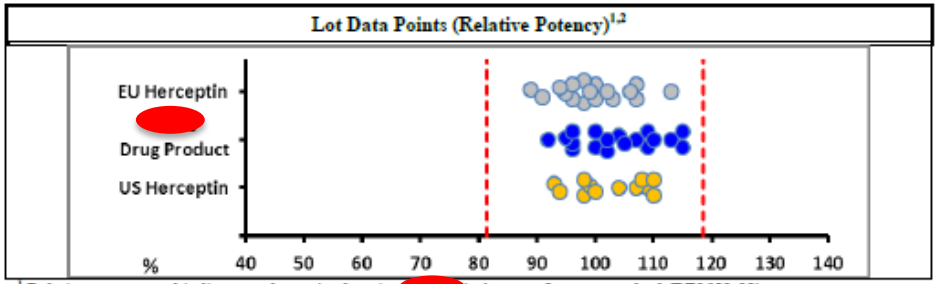
[1] Relative potency or binding was determined against [redacted] *in-house* reference standard (RF0650-02)
[2] The dotted red line represents the limits based on mean ± 3SD range of EU-approved Herceptin® lots

Figure 3.2.R.4-33: Scatter Plot for HER2 Binding Affinity (ELISA) Results



[1] Relative potency or binding was determined against CT-P6 *in-house* reference standard (RF0650-02)
[2] The dotted red line represents the limits based on mean ± 3SD range of EU-approved Herceptin® lots

Figure 3.2.R.4-37: Scatter Plot for *In Vitro* Bioactivity Results



[1] Relative potency or binding was determined against [redacted] *in-house* reference standard (RF0650-02)
[2] The dotted red line represents the limits based on mean ± 3SD range of EU-approved Herceptin® lots

Figure 3.2.R.4-39: Scatter Plot for C1q Binding Affinity Results

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 December 2014
EMEA/CHMP/BMWP/42832/2005 Rev1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues

Date for coming into effect	01 July 2015

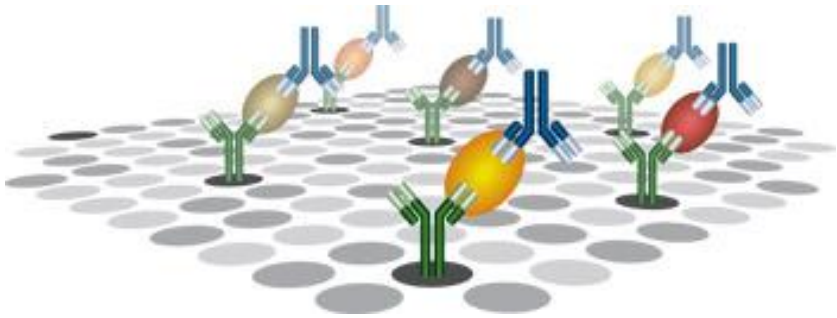
NON CLINICAL STUDIES

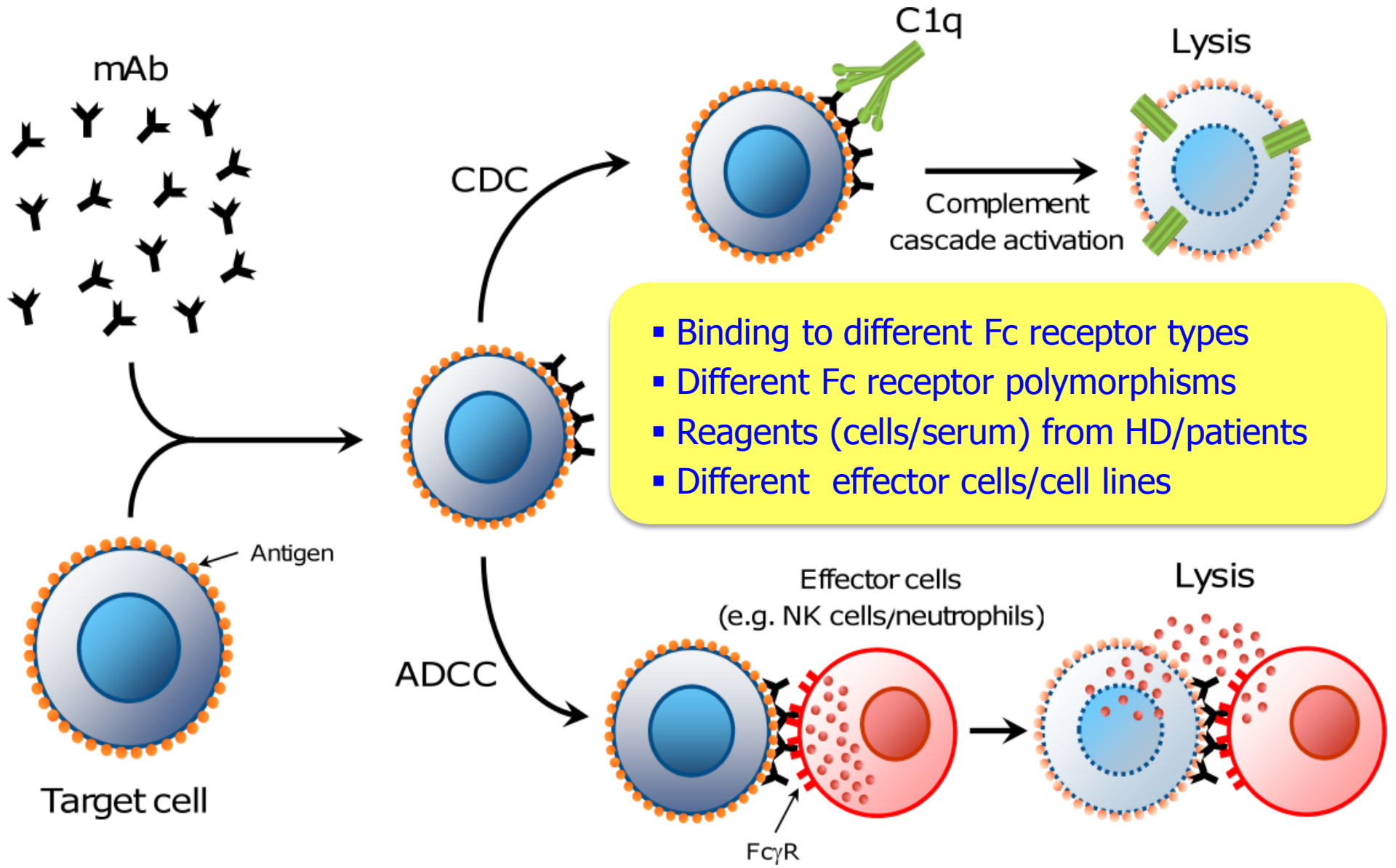
Step by step approach:

① *in vitro* studies

② in vivo studies needed?

③ *in vivo studies*





- Binding to different Fc receptor types
- Different Fc receptor polymorphisms
- Reagents (cells/serum) from HD/patients
- Different effector cells/cell lines

Quality Attribute		MabThera®	CT-P10 Drug Product	Rituxan®
	G0 (%)	0.8 ± 0.2	0.4 ± 0.1	0.8 ± 0.2
Sialic Acid Analysis	Molar Ratio (sialic acid / protein mol / mol)	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.02
Monosaccharide Analysis (Molar Ratio)	Fuc	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
	GlcNAc	5.6 ± 0.2	5.5 ± 0.2	5.6 ± 0.3
	Gal	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.1
	Man	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0.2
Biological Analysis (N = 15)				
Cell-based CD20 Binding Affinity (CELISA) (%)		96 ± 6.2	97 ± 5.8	96 ± 7.0
CDC (%)		100 ± 5.3	100 ± 3.8	99 ± 3.3
ADCC (%)		97 ± 4.3	97 ± 6.0	98 ± 4.0
Apoptosis (FACS) (%)		101 ± 5.1	103 ± 3.1	99 ± 5.0
C1q Binding Affinity (ELISA) (%)		104 ± 6.1	104 ± 7.8	105 ± 4.8
FcγRIIIa-V Binding Affinity (SPR) (%)		104 ± 6.6	100 ± 3.3	103 ± 6.9
FcγRIIIa-F Binding Affinity (SPR) (%)		108 ± 8.6	100 ± 2.5	105 ± 8.9
FcγRIIIb Binding Affinity (SPR) (%)		105 ± 8.5	102 ± 7.7	101 ± 8.8
FcγRIIa Binding Affinity (SPR) (%)		100 ± 3.0	99 ± 3.3	100 ± 3.3
FcγRIIb Binding Affinity (SPR) (%)		97 ± 5.6	98 ± 7.1	94 ± 6.7
FcγRI Binding Affinity (SPR) (%)		101 ± 3.2	100 ± 3.0	100 ± 2.6
FcRn Binding Affinity (SPR) (%)		100 ± 2.3	101 ± 2.4	100 ± 2.2
Biological Analysis Supporting to Extrapolation (N = 3)				
Apoptosis using MEC-2 B cells (B-CLL cell line) as target cells		103 ± 2.5	102 ± 3.5	103 ± 4.6
CDC using B cells purified from healthy donor PBMCs as target cells		101 ± 0.6	103 ± 2.6	101 ± 2.6
CDC using B cells purified from NHL patient PBMCs as target cells		101 ± 4.2	101 ± 0.6	100 ± 1.0
CDC using B cells purified from CLL patient PBMCs as target cells		102 ± 7.5	104 ± 5.3	103 ± 6.0
ADCC using B cells purified from healthy donor PBMCs as target cells		97 ± 6.1	99 ± 4.0	100 ± 4.5
ADCC using B cells purified from NHL patient PBMCs as target cells		98 ± 1.0	98 ± 1.5	102 ± 2.9
ADCC using B cells purified from CLL patient PBMCs as target cells		100 ± 1.7	99 ± 3.1	103 ± 1.0
ADCP using B cells purified from healthy donor PBMCs as target cells		102 ± 4.7	100 ± 4.0	102 ± 2.5
ADCP using B cells purified from NHL patient PBMCs as target cells		101 ± 6.6	97 ± 6.4	98 ± 6.6
ADCP using B cells purified from CLL patient PBMCs as target cells		101 ± 5.3	102 ± 3.0	102 ± 1.2

[1] Values are presented as mean ± standard deviation

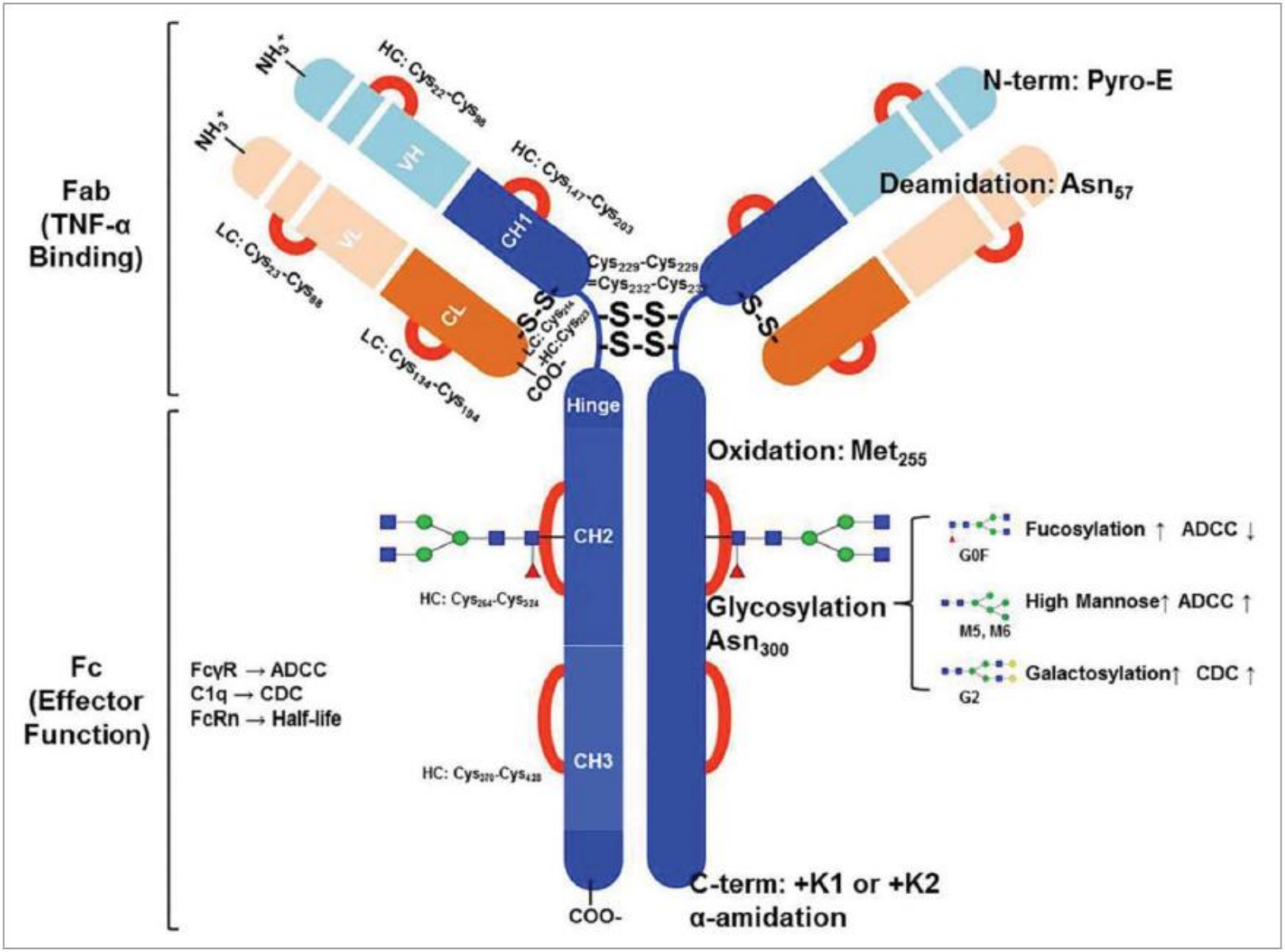


Figure 1. Schematic Structure of Infliximab.

Step 1: PK/PD studies

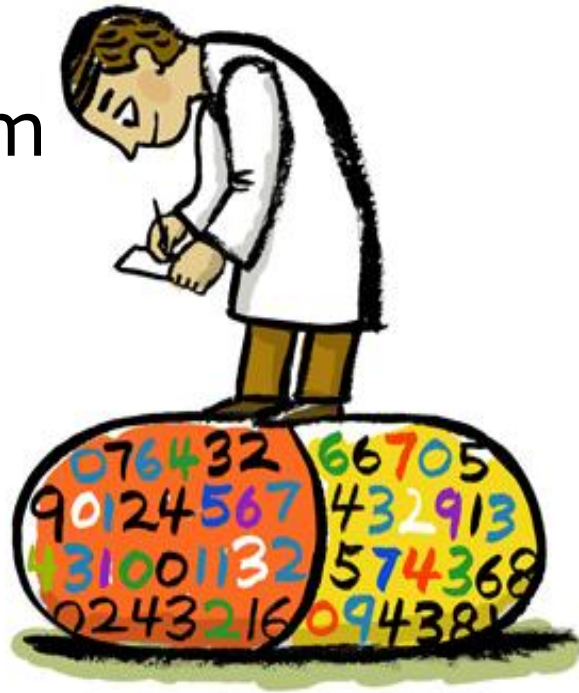
Step 2: Efficacy & Safety

Primary objective:

to show comparable PK in a sufficiently sensitive and homogeneous population.

Healthy volunteers OK
(patients, if a toxic mechanism of action)

PK studies



PD STUDIES

Use of **multiple PD markers** recommended (if possible)

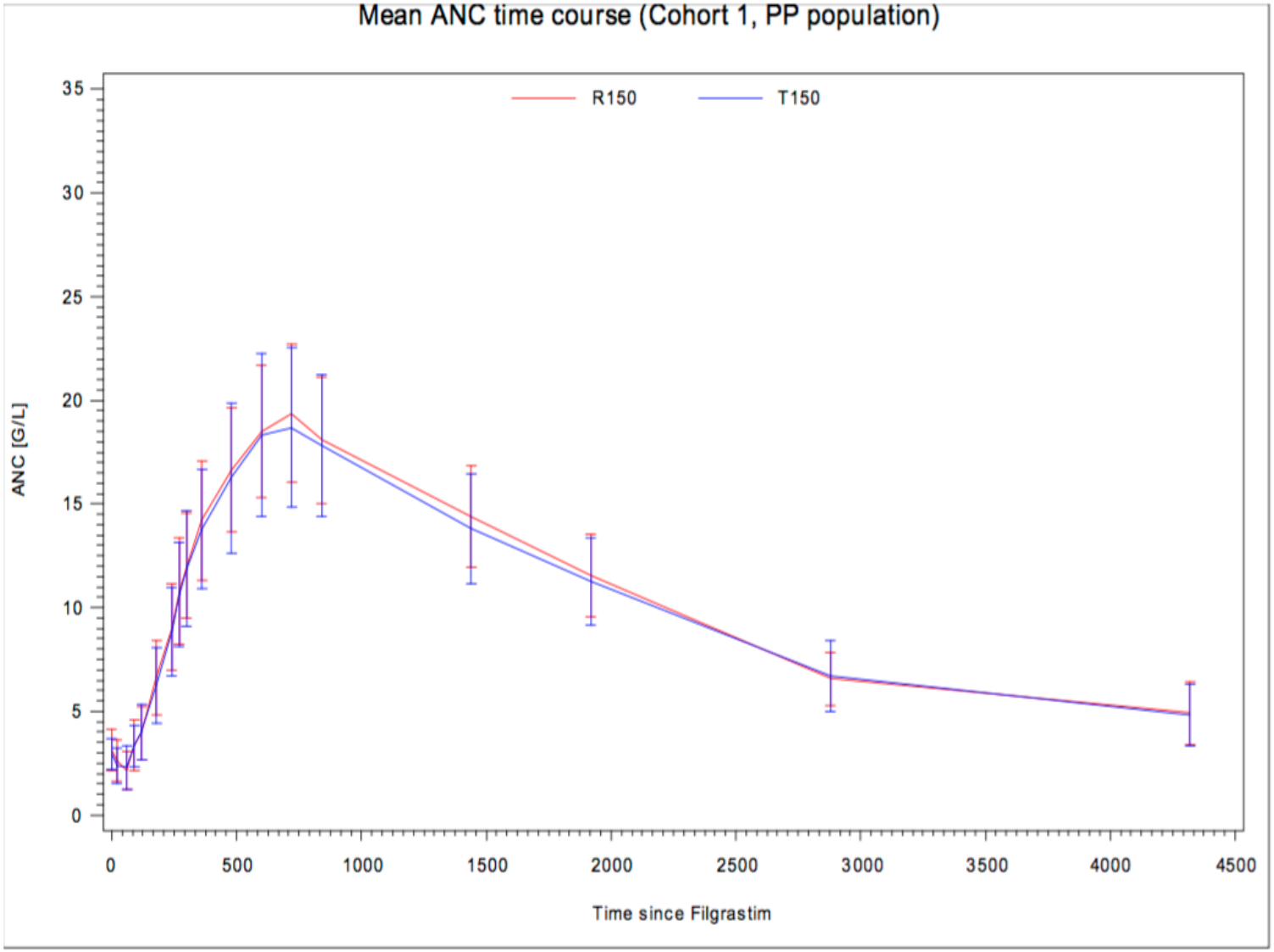
Explore dose-concentration-response or time-response relationships

PD markers as **pivotal evidence** for comparable efficacy if:

- Clear dose-response relationship shown
- At least one PD marker is an accepted surrogate marker and can be related to patient outcome

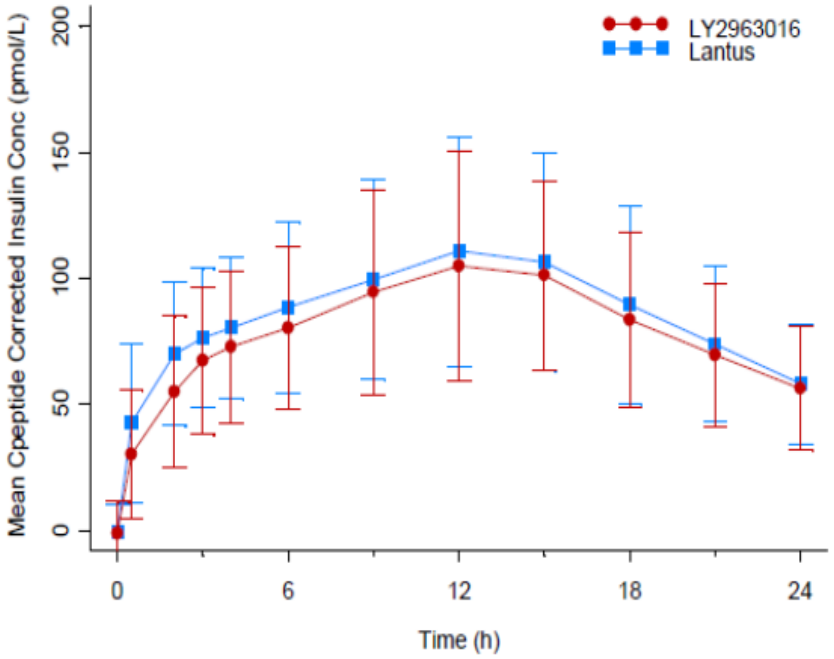
Absorption and Distribution

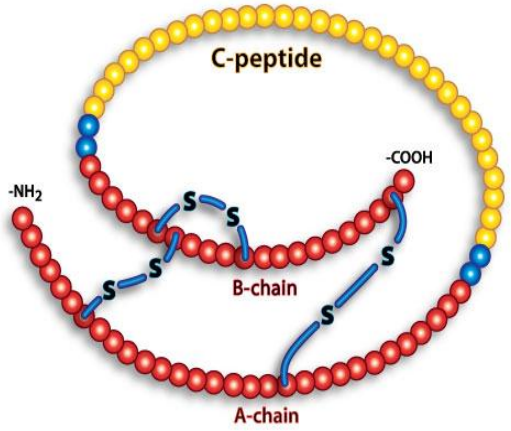
Figure 02: Study KWI-300-102: Mean ANC-Time Profile Following a Single Subcutaneous Injection of 150 µg of Apo-Filgrastim or Neupogen to Healthy Male and Female Volunteers (PP-Population)

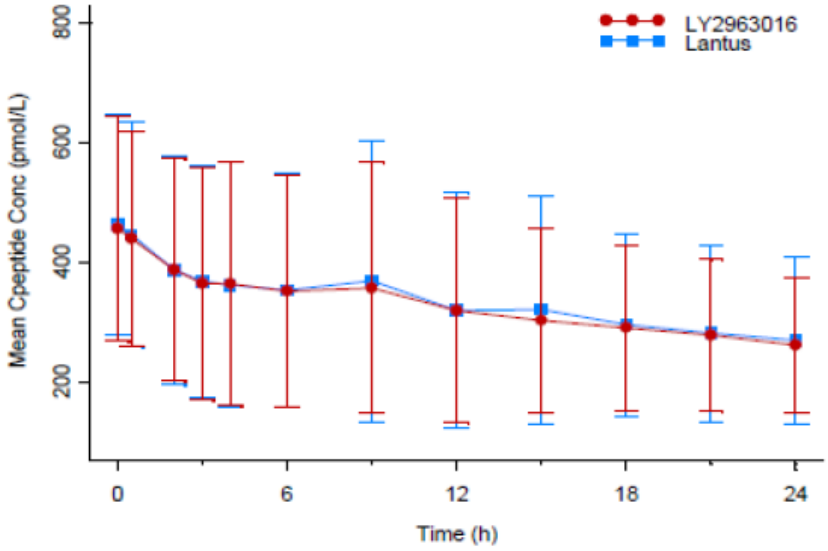


T150 = Apo-Filgrastim 150µg, R150 = Neupogen 150µg

Figure 5: PK & PD profiles in pivotal study ABEA (healthy volunteers; 0.5 U/kg)







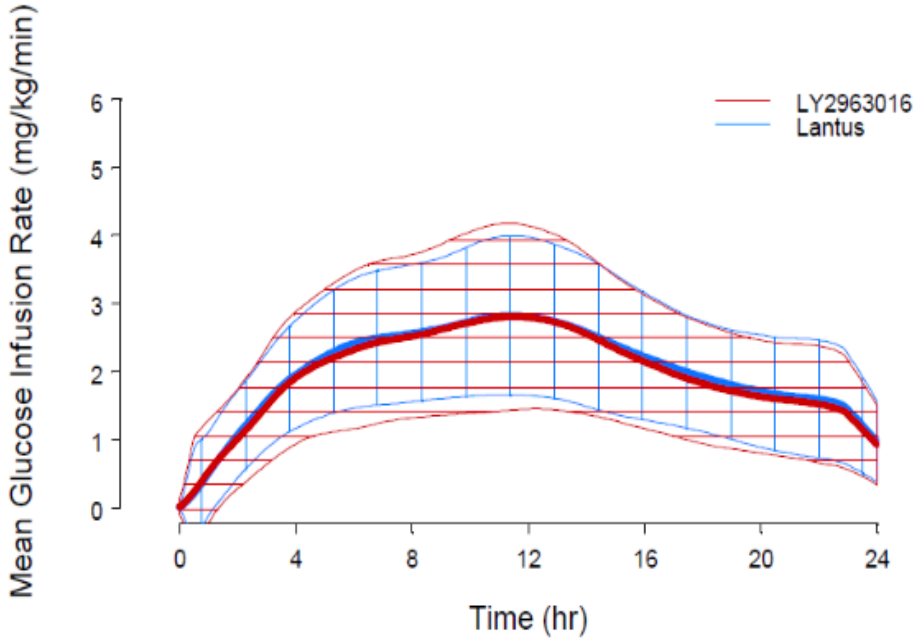
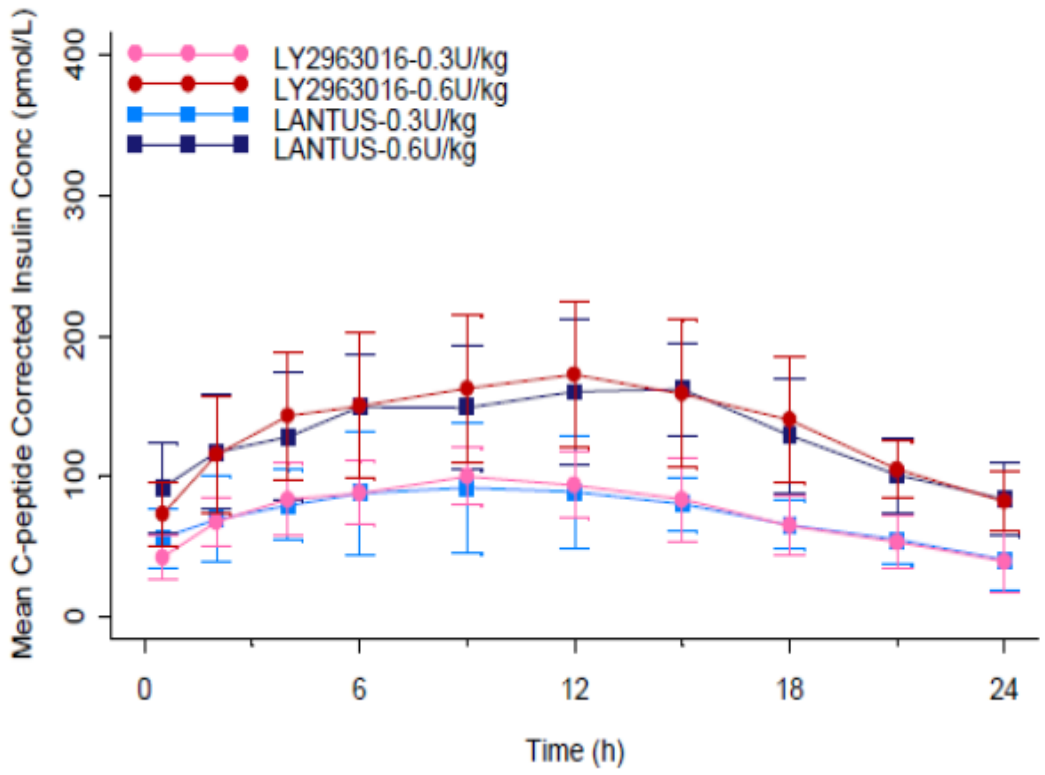
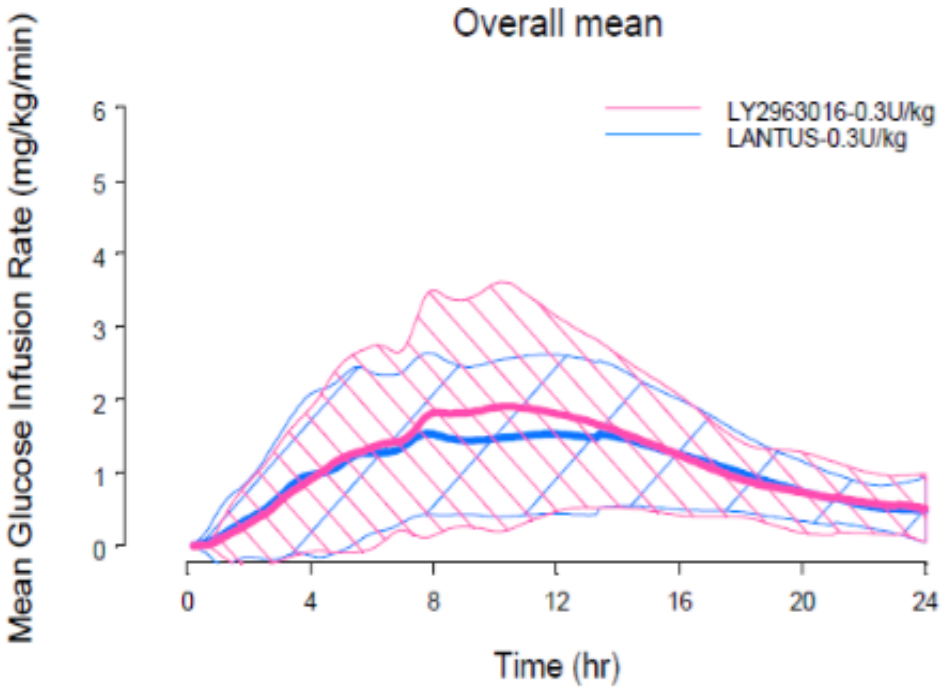
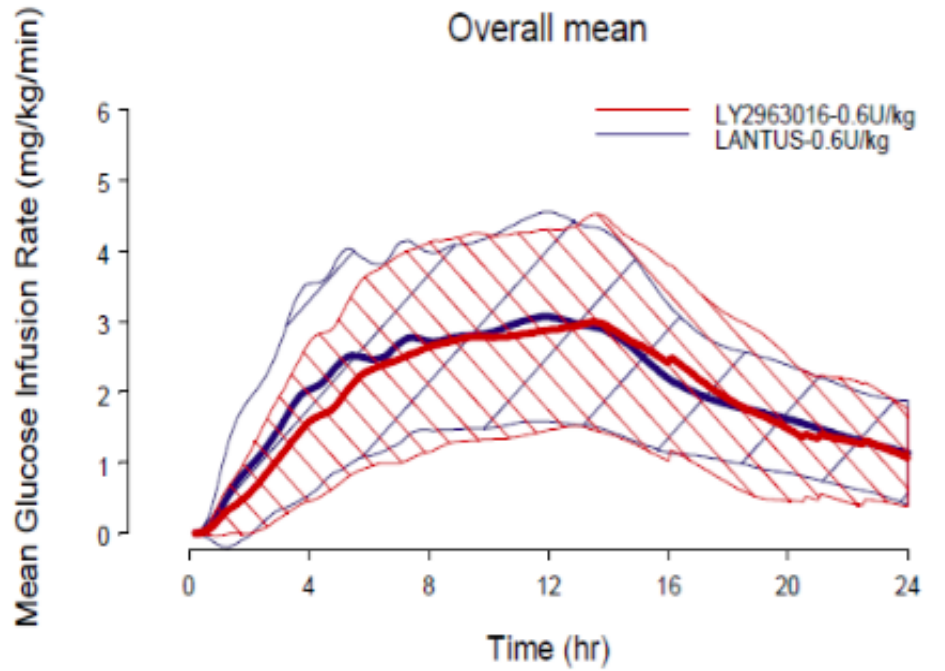


Figure 6: PK & PD profiles in study ABEM (healthy volunteers; 0.3 & 0.6 U/kg)




Overall mean
LY2963016-0.3U/kg
LANTUS-0.3U/kg
Mean Glucose Infusion Rate (mg/kg/min)
0
1
2
3
4
5
6
0
4
8
12
16
20
24
Time (hr)


Overall mean
LY2963016-0.6U/kg
LANTUS-0.6U/kg
Mean Glucose Infusion Rate (mg/kg/min)
0
1
2
3
4
5
6
0
4
8
12
16
20
24
Time (hr)

Efficacy & Safety

- Adequately powered, randomised, parallel group comparative clinical trial, preferably double-blind, normally equivalence trials.
- Deviation from relevant efficacy guidelines should be justified
- Most sensitive patient population and clinical endpoints are preferred
- Comparative S data (immunogenicity)
- Extrapolation of indications is possible; will be based on the overall evidence of comparability

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 December 2014
EMEA/CHMP/BMWP/42832/2005 Rev1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues

6. Extrapolation of indications

Extrapolation of clinical efficacy and safety data to other indications of the reference mAb, not specifically studied during the clinical development of the biosimilar mAb, is possible based on the overall evidence of comparability provided from the comparability exercise and with adequate justification. If pivotal evidence for comparability is based on PD and for the claimed indications different mechanisms of action are relevant (or uncertainty exists), then applicants should provide relevant data to support extrapolation to all claimed clinical indications. Applicants should support such extrapolations with a comprehensive discussion of available literature including the involved antigen receptor(s) and mechanism(s) of action.

Extrapolation of data is already an established scientific and regulatory principle that has been exercised for many years, for example, in the case of **major changes** in the manufacturing process of **originator biologicals**.

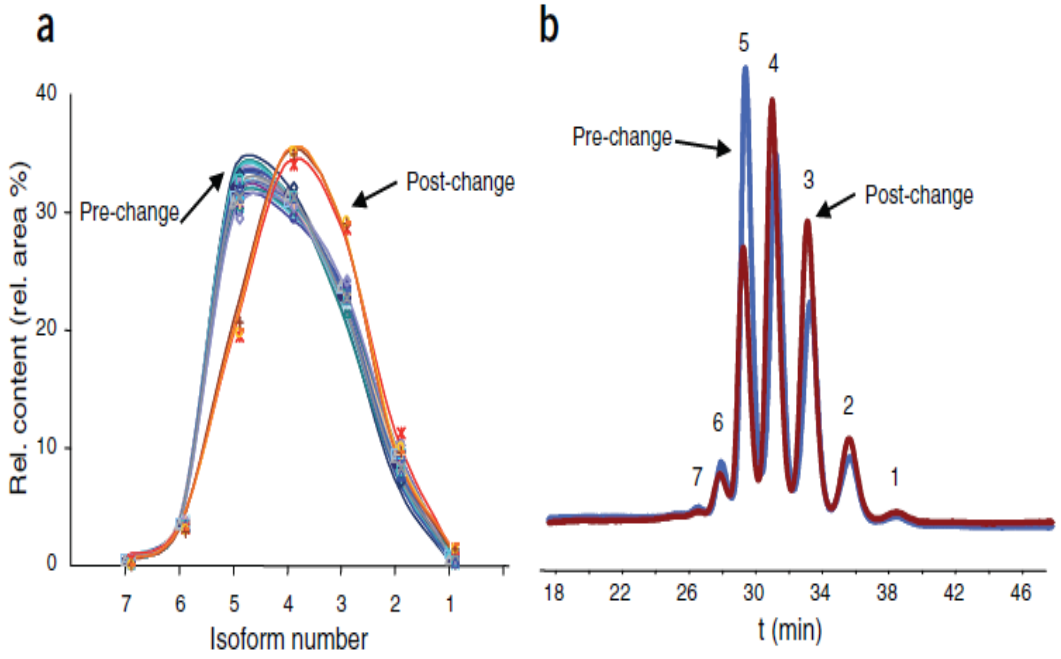


Figure 1 Comparison of the pre- and post-change Aranesp batches measured by capillary zone electrophoresis. (**a**) Relative content of the individual isoforms of the pre-change ($n = 18$) and the post-change ($n = 4$) batches. (**b**) Representative electropherograms; peaks are labeled with the isoform number.

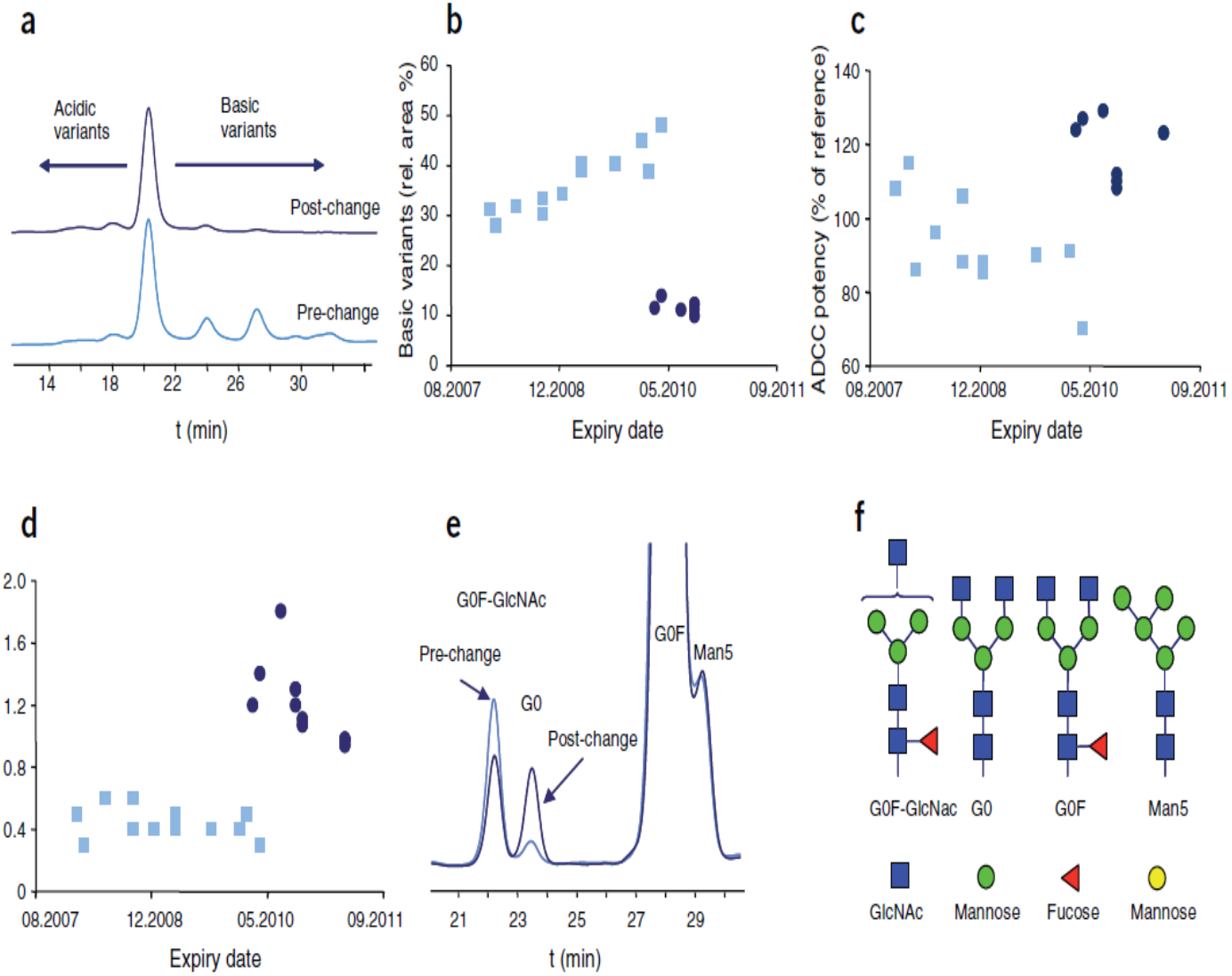


Figure 2 Comparison of the different pre- and post-change batches of Rituxan/Mabthera. (**a**) Exemplary CEX chromatograms. (**b**) Amount of basic variants of the pre-change ($n = 12$) and post-change ($n = 6$) batches as measured by CEX. (**c**) ADCC potency of the pre-change ($n = 11$) and post-change ($n = 8$) batches. (**d**) Relative amount of the G0 glycan of the pre-change ($n = 13$) and post-change ($n = 11$) batches. (**e**) Exemplary glycan mapping chromatogram (**f**) Glycan legend.

Another example where extrapolation has already been accepted is the introduction of a new subcutaneous (SC) formulation of a hitherto intravenously (IV) applied product. Although the formulation and bioavailability of the SC product will be different, one clinical study is usually sufficient to grant several, if not all, clinical indications approved for the IV product. This is illustrated by the recent approval of an SC formulation of an anti-Her2 monoclonal antibody (mAb) based on clinical data in the neoadjuvant setting, which were extrapolated to the metastatic setting based on the totality of the evidence from all data provided.[27] It is notable that the SC formulation of that antibody contains recombinant human hyaluronidase (rHuPH20) as a permeation enhancer and is thus considerably different from the IV formulation. A formulation difference of this magnitude would not be acceptable for a biosimilar compared with the reference product.

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2nd Turkish Interactive Workshop on
REGULATORY ASSESSMENT OF BIOSIMILARS
24 September 2019